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Separation of human eosinophils in density gradients of polyvinylpyrrolidone-coated silica gel (Percoll)

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Summary. A method for isolation of eosinophils from human peripheral blood using isosmolar solutions of polyvinylpyrrolidone-coated silica gel (Percoll) is described. The purity ranged from 86 to 99% eosinophils in the final preparation and the recovery was 38–56%. The separation technique did not affect the viability or the metabolic capacities of the cells.

INTRODUCTION

There are several methods reported for the isolation of eosinophils, most of them based on density gradient centrifugations. The eosinophil has the highest density of all leucocytes and therefore lends itself to this type of separation technique. Density gradients of bovine serum albumin (Alexander & Spriggs, 1962), sodium metrizoate (Day, 1970) and sodium diatrizoate (Gleich & Loegering, 1973; Mahmoud, Kellermeyer & Warren, 1974) have the disadvantage of being hypertonic altering the density of the cells applied to the gradients. For instance the excellent separation of neutrophils in Isopaque–Ficoll as described by Böyum (1968) is based on this fact since neutrophils separated in isosmolar density gradients of Isopaque–Ficoll show an altered density distribution profile (Wells, Opelz & Cline, 1977). In this communication, the isolation of highly purified and metabolically intact eosinophils from density gradients of polyvinylpyrrolidone-coated silica gel is described

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MATERIALS AND METHODS

Collection of blood

Heparinized venous blood was collected from healthy volunteers. Five parts of blood was mixed with one part of 6% dextran (Dextran 250, Pharmacia Fine Chemicals, Uppsala, Sweden) in 0·15 M NaCl and left at room temperature for 40–45 min to let the red cells sediment. The dextran-plasma was collected and centrifuged at 450 *g* for 8 min. The cells were washed once in saline and suspended in Percoll solution (density 1·070 g/ml) with 5% foetal calf serum (FCS). Cell concentration was adjusted to 25–30 × 10^6/ml.

Density gradient centrifugation

Nine parts of Percoll (Pharmacia Fine Chemicals) were mixed with one part of Hanks's BSS 10× (ten times physiological concentration), called heavy solution. One part of heavy solution was mixed with nine parts of Hanks's BSS 1×; this mixture is called light solution.

Density was determined by refractometer readings from a standard curve made from different dilutions of Percoll of known density determined by weighing 10 ml portions in pyknometers. Heavy and light solutions were then mixed to obtain mixtures of the following densities: 1·100, 1·090, 1·085, 1·080 and 1·070 g/ml,

according to the formula given by Day (1970). The solutions were stored in rubber-capped bottles at +4° and incubated in open tubes for 1 h in a CO_2-incubator (5% CO_2) for adjustment of pH before the gradients were made. Gradients were formed using a peristaltic pump (LKB 2120 Varioperpex II pump, LKB-Beckman Instruments, Bromma, Sweden) and consisted of 1·5 ml 1·100, 3 ml 1·090, 3 ml 1·085 and 3 ml 1·080 g/ml in 17 × 100 mm polypropylene tubes (Falcon Plastics, Los Angeles, CA). The cells suspended in Percoll 1·070 g/ml supplemented with 5% FCS were layered on top of the gradients, 2 ml per gradient. The tubes were then centrifuged in an angle rotor (angle 45°) at 1600 *g* for 20 min at room temperature. One-millilitre fractions were collected from the bottom of the tubes using the peristaltic pump. The density of each fraction was determined and the cells were washed twice in KRP buffer (Ca-free) before counting in a haemocytometer. Cytocentrifuge smears were prepared from each fraction for differential counts.

Metabolic activity of isolated eosinophils

Fractions with the highest purity of eosinophils were pooled and used to measure the metabolic activity of the cells. Oxygen consumption during phagocytosis was assayed principally as described in (Olofsson, Odeberg & Olsson, 1976). 2–8 × 10^6 cells in 2·24 ml were challenged with heat-killed yeast cells at a ratio of 10:1 and oxygen consumption was followed over a 5 min period. The ability of isolated eosinophils to iodinate heat-killed yeast cells was tested as described previously (Olsson, Olofsson & Odeberg, 1972). 1·5–10 × 10^5 cells in 0·5 ml were mixed with heat-killed yeast cells (ratio 50:1). Resting cells were incubated also.

The reaction was terminated after 30 min of incubation by addition of 0·1 ml sodium thiosulphate (0·1 M) and 4·5 ml cold TCA (10%). The TCA precipitable material was washed twice in TCA and the radioactivity measured in a gamma-spectrometer. For comparison neutrophil-enriched fractions (> 97% neutrophils) of the gradients were used under the same conditions as the eosinophils. Viability of the cells was estimated by trypan blue exclusion.

RESULTS

Percoll easily forms linear gradients during centrifugation. The procedure described in this paper resulted in density gradients linear between 1·078 and 1·093 g/ml

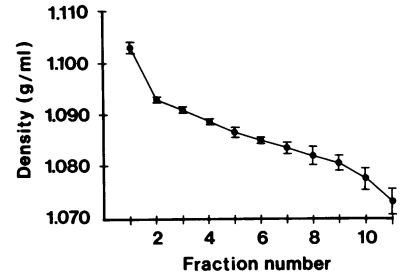

Figure 1. Density profile of Percoll gradients. Results are mean ± SD of four gradients made on different occasions. The mean SD in all fractions was 0·00106 g/ml.

(Fig. 1) with a very high reproducibility (mean SD = 0·00106 g/ml in all fractions). Figure 2 shows the separation of eosinophils, neutrophils and mononuclear cells (monocytes and lymphocytes) in two representative cases. The highest purity of eosinophils was found at 1·090–1·095 g/ml. Pooled fractions within the range of 1·086–1·100 g/ml (four cases) showed 86–99% eosinophils with a recovery of 38–56% of the eosinophils applied to the gradient. Neutrophils were found with the highest purity (92–99%) at 1·080–1·085 g/ml whereas mononuclear cells were concentrated in the upper region of the gradient around 1·070 g/ml. The majority of the neutrophils found at 1·090–1·100 g/ml were senescent cells with dense pyknotic nuclei. Basophilic granulocytes were found exclusively between 1·070 and 1·080 g/ml.

Figure 3 shows the density distribution (mean ± SEM in percentage of all recovered eosinophils) and

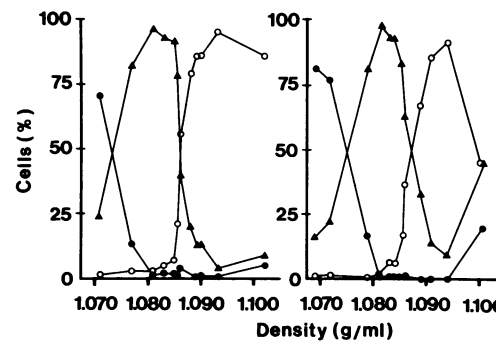

Figure 2. Separation of eosinophils (o), neutrophils (▲) and monocytes and lymphocytes (●) in Percoll gradients in two representative cases. The values plotted are the differential counts of each fraction and shows the purity of the cells.

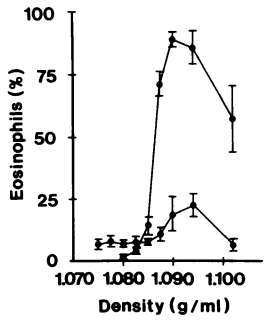

Figure 3. Density distribution profile (lower curve, $n=4$, mean ± SEM in percentage of all recovered eosinophils) and purity (upper curve, $n=5$, mean ± SEM in percentage eosinophils). Each point represents one fraction.

purity of eosinophils in four and five cases respectively. The inter-individual variation was small with the eosinophils peaking between 1·090 and 1·094 g/ml and with the highest purity at 1·090 g/ml showing 90% ± 2·6 (SEM) eosinophils. Erythrocytes remaining in the dextran–plasma applied to the gradient aggregated during centrifugation and were recovered at densities above 1·090 g/ml. They were, however, easily removed by lysis in 0·87% ammonium chloride for 5 min. Viability of isolated eosinophils was excellent as inferred from trypan blue exclusion tests; never more than 3% of the cells were stained.

To show that the separation procedure did not destroy the metabolic capacities of the eosinophils, their oxygen consumption and the ability to iodinate heat-killed yeast cells were measured. Neutrophils obtained from the same gradients were tested in parallel experiments for comparison. In five experiments, the eosinophils consumed 3·06 ± 0·48 (mean ± SD) μl O_2/min/10^7 cells when challenged with heat-killed yeast cells. Neutrophils consumed 2·38 ± 0·74 μl O_2/min/10^7 cells. Resting eosinophils incorporated 1·96 ± 1·4 (mean ± SD; $n=4$) nmol I^-/h/10^6 cells into TCA-precipitable material, which was approximately fourteen times more than resting neutrophils showing 0·14 ± 0·05 nmol I^-/h/10^6 cells. When the eosinophils were challenged with yeast cells they increased I^- incorporation 1·5–3 times to 3·61 ± 1·75 (mean ± SD; n=4) nmol I^-/h/10^6 cells. Neutrophils showed 3·14 ± 0·29 nmol I^-/h/10^6 cells. These experiments clearly indicated that the eosinophils retained their metabolic integrity through the separation procedure.

DISCUSSION

Percoll consists of colloidal silica particles coated with polyvinylpyrrolidone and has several properties that renders it suitable for separation of living cells. Its low osmolality and low viscosity make it possible to obtain density gradients with physiological osmolality and rapid separation. It has been successfully used in separation of rat liver cells (Pertoft & Laurent, 1977), HeLa cells and viruses (Wolff & Pertoft, 1972), bone marrow cells (Olofsson, Gärtner & Olsson, 1980) and subcellular components (Pertoft & Laurent, 1977) without affecting the properties of the material separated.

The density gradient separation described here has the advantage over other density gradients to be performed at physiological osmolality avoiding the adverse effects hypertonicity may have on cell function. Purity and recovery is of the same magnitude as the best methods described previously for isolation of human eosinophils of normal peripheral blood. Day (1970) used hypertonic sodium metrizoate and obtained 93% pure eosinophils with about 52% recovery, which should be compared to 86–99% purity and 38–56% recovery obtained with Percoll.

The separation also has the advantage of giving pure neutrophils from the same gradient, which is valuable in comparative studies of eosinophils and neutrophils.

Functional studies of eosinophils isolated in Percoll demonstrated intact metabolic activity. The observations on oxygen consumption during phagocytosis and iodination in resting and phagocytosing cells are in excellent agreement with the results obtained by DeChatelet, Shirley, McPhail, Huntley, Muss & Bass (1977). Isolation of eosinophils from density gradients of Percoll should be valuable in studies of eosinophil function and eosinophil granule contents.

Note added in proof

Some batches of Percoll and HBSS will result in slightly hyperosmolar solutions disturbing the separation by increasing the density of neutrophils. This is easily avoided by using the following formula for heavy solution: 90 ml Percoll, 8 ml HBSS 10× and 2 ml water.

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REFERENCES

ALEXANDER R.F. & SPRIGGS A.L. (1962) A method for obtaining concentrates of eosinophils from blood. *J. clin. Path.* **15,** 188.

BÖYUM A. (1968) Separation of leucocytes from blood and bone marrow *Scand. J. clin. Lab. Invest.* **21,** Suppl. 97,77.

DAY R.P. (1970) Eosinophil cell separation from human peripheral blood. *Immunology,* **18,** 955.

DECHATELET L.R., SHIRLEY PAMELA S., MCPHAIL LINDA C., HUNTLEY CAROLYN C., MUSS H.B. & BASS D.A. (1977) Oxidative metabolism of the human eosinophil *Blood,* **50,** 525.

GLEICH G.J. & LOEGERING D.A. (1973) Selective stimulation and purification of eosinophils and neutrophils from guinea-pig peritoneal fluids *J. Lab. clin. Med.* **82,** 522.

MAHMOUD A.A.F., KELLERMEYER W.R. & WARREN K.S. (1974) Production of monospecific rabbit anti-human eosinophil serum. *New Engl. J. Med.* **290,** 417.

OLOFSSON T., ODEBERG H. & OLSSON I. (1976) Granulocyte function in chronic granulocytic leukemia. II. Bactericidal capacity, phagocytic rate, oxygen consumption and granule protein composition in isolated granulocytes. *Blood,* **48,** 581.

OLOFSSON T., GÄRTNER INGRID & OLSSON I. (1980) Separation of human bone marrow cells in density gradients of polyvinylpyrrolidone coated silica gel (Percoll). *Scand. J. Haemat.* (In press.)

OLSSON I., OLOFSSON T. & ODEBERG H. (1972) Myeloperoxidase mediated iodination in granulocytes. *Scand. J. Haemat.* **9,** 483.

PERTOFT H. & LAURENT T.C. (1977) Isopycnic separation of cells and organelles by centrifugation in modified silica gradients. In: *Methods of Cell Separation* (Ed. by N. Catsimpoolas), p. 25. Plenum Publishing Co, New York.

WELLS J.R., OPELZ G. & CLINE M. (1977) Characterization of functionally distinct lymphoid and myeloid cells from human blood and bone marrows. I. Separation by a buoyant density gradient technique. *J. immunol. Meth.* **18,** 63.

WOLFF D.A. & PERTOFT H. (1972) Separation of HeLa cells by colloidal silica density gradient centrifugation. *J. Cell Biol.* **55,** 579.